(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 August 2005 (18.08.2005)

PCT

(10) International Publication Number
WO 2005/074470 A3

(51) International Patent Classification[7]: C12N 15/09, 15/64

(21) International Application Number: PCT/US2004/043139

(22) International Filing Date: 23 December 2004 (23.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/533,943 2 January 2004 (02.01.2004) US

(71) Applicant *(for all designated States except US)*: **SAINT LOUIS UNIVERSITY** [US/US]; Saint Louis University Technology, Transfer Office, 3556 Caroline Mall, Suite C110, Saint Louis, MO 63104 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **YACIUK, Peter** [US/US]; 130 S. Gore Street, Saint Louis, MO 63119 (US).

(74) Agent: **ZAHNER, Joseph**; Saint Louis University, 3556 Caroline Mall, Suite C110, Saint Louis, MO 63104 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 8 December 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: KITS AND METHODS FOR MAKING LARGE RECOMBINANT POLYNUCLEOTIDES

(57) Abstract: Cloning of manipulated polynucleotide sequences is time consuming, labor intensive and inefficient, especially when polynucleotide sequences are long. Disclosed are methods and kits for manipulating and cloning large polynucleotide sequences that are both efficient and easy to use relative to previously described methods and kits. The disclosed invention utilizes polynucleotide fragments with mutually non-complementary asymmetric single-stranded sticky ends, which may be manipulated to produced desired sequences, then allowed to reassemble in vitro into a useful recombinant polynucleotide. Additionally, an excess of synthetic double-stranded oligonucleotides or PCR generated polynucleotides, which contain single-stranded sticky ends that are compatible with the sticky ends of the polynucleotide fragments derived from a parent polynucleotide, may be added to the polynucleotide fragments to compete with those polynucleotide fragments for ligation into a full-length recombinant polynucleotide product.

WO 2005/074470 A3